

On the Bionomics and Post-Embryonic Development of certain Cynipid Hyperparasites of Aphides.

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With 11 Text-figures.

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INTRODUCTION.

THE biology of the entomophagous Cynipidae, which include the sub-families of *Encoilinae*, *Figitinae*, and *Charipinae*, has been little studied. The *Encoilinae* and *Figitinae* are known to be parasitic chiefly upon Diptera. The *Charipinae* have hitherto been reared from Aphididae, and occasionally from Coccidae: but no account of their development has been published, and systematic workers have described them indifferently as parasites or hyperparasites. It is probable that the latter view will prove correct for the majority of the sub-family.¹

The following is an account of the bionomics of certain of these Cynipidae, of the genus *Charips*. This was formerly known as *Allotria*, but in 1910, Kieffer (19) reverted to the name originally given by Haliday in 1870, and his terminology has been followed here. The genus is divided into two sub-genera, *Bothrioxysta*, Kieff., and *Charips*, Hal. The majority of individuals reared from material collected in the field in the course of this work were of the species *Bothrioxysta curvata*, Kieff.; but a few examples of *Charips victrix*, Westw., and of another genus, *Alloxysta erythrothorax*, Hartig, were obtained. No distinction was observed between the larval forms, which is not surprising where the specific distinctions of the adults are variable and slight. It is even possible that certain forms, now ranking as species, may not be physiologically distinct; for in one instance, in captivity, a male of *Alloxysta erythrothorax* appeared to mate with a female of *Charips victrix*, which afterwards oviposited.

Hence throughout this work it has been thought most convenient to use the generic name, *Charips*, when speaking

¹ Silvestri (23), in a foot-note to his work on *Encyrtus aphidivorus*, remarks that *Allotria* (*Charips*) is a hyperparasite of aphides through *Aphidius* (Braconidae); and he adds that it lives upon the host internally, an observation which has been neglected by writers, both before and since.

generally, and to indicate the particular sub-genus or species where necessary.

I would here express my sincere thanks to Professor J. Stanley Gardiner for giving me facilities to carry out the work in the Zoological Laboratory at Cambridge; and my obligations to Professor J. J. Kieffer, who kindly determined the examples of Cynipidae submitted to him.

MATERIAL.

The material used was obtained in Cambridge in the summer of 1920. *Charips* (*Allothria*) has been reared from various Aphidiidae in different aphides, but throughout this work, *Aphidius ervi*, Hal., a parasite of *Macrosiphum urticae*, Kalt., was used, as the comparatively large size of the cocoons rendered them convenient for dissection. The parasite and its host were common and widely distributed round Cambridge in June and July. Moreover, the food plant of this aphid, the common nettle, usually grew in isolated patches along the roadside. This was an advantage, since the *Aphidius*, after parasitization by the Cynipid, is liable to secondary parasitization by certain ecto-parasitic Chalcids and Proctotrypids, which kill both the host and the first hyperparasite. Collections made from one spot showed that almost every *Aphidius*, whether attacked by a Cynipid or not, might bear one or more of these external parasites; while collections made fifty yards away were free from secondary infestation, and contained Cynipid larvae in all stages of development.

The rearing methods were the same as those used when studying *Lygocerus* (10). Camera lucida drawings and measurements were made from living specimens, mounted in salt solution or dilute glycerine. The larva, and the host when necessary, were also studied in serial sections.

BIOLOGICAL NOTE ON THE HOST.

The development of the Braconid, *Aphidius*, within the aphid has been described by Seurat (21), Timberlake (25), and others.

The egg is deposited in the haemocoel of the host, and in the course of development a pseudo-serosa or trophic membrane of hypertrophied cells is formed round the embryo. The first larval stage is a transparent caudate form, which varies somewhat in different genera, the cauda of *Aphidius* being single, whereas, according to my observations, it is bifid in *Ephedrus* and *Praon*. This appendage diminishes in the succeeding instars, and the larva, which lies curved head to tail in the body of the host, gradually assumes the apodous maggot-shaped form usual among hymenopterous larvae. At first the presence of the parasite makes little difference to the aphid, which feeds and reproduces as usual; but, as development proceeds, degeneration of the host's tissues sets in. The embryos are affected first, and then the fat-body. The 'pseudo-vitellus' or symbiotic organ is not attacked until a later stage, and the nervous system and alimentary canal remain unchanged until just before the *Aphidius* transforms. When they, in common with the rest of the fluids of the body, are ingested by the parasite. The tissues break down into large globules, which in stained preparations appear as a vacuolated mesh-work of connective tissue containing droplets of fat, while there is often a mass of degenerating nucleoplasm in the centre of the mass. By what means the parasite thus breaks down the surrounding tissues is not known, but although the larva possesses powerful mandibles, chemical rather than mechanical action seems probable.

As soon as the *Aphidius* has completely emptied the body of the aphid, it changes apneustic for peripneustic respiration, and weaves a cocoon inside the dry skin with silk secreted by the salivary glands.

The meconium is then voided and metamorphosis takes place.

PAIRING.

In *Bothrioxysta curvata*, reproduction was either sexual or parthenogenetic according to whether a male was introduced into the rearing-tube or not. All observed ovipositions of *Charips victrix* took place after mating, but the ovipositions of *Alloxysta* were not determined.

OVIPOSITION.

The female *Charips* oviposits in the *Aphidius* larva only while the aphid is alive. In this it differs from other hyperparasites, such as *Lygocerus* (Proctotrypidae) and *Asaphes* (Chalcidae), which insert their eggs only after the host has woven its cocoon. My observations in this respect are opposed to those of Gatenby (8), who says: 'The Cynipid parasitic forms associated with aphids apparently never attack live Aphidae, but seek out the dried skins of those already parasitized by an *Aphidius*.'

Subject to the condition that the Braconid larva shall still be bathed in the body fluids of its aphid host, the Cynipid has considerable latitude in its choice of a victim. The *Aphidius* usually selected is in the third or early fourth instar, but a second instar larva may be chosen (Text-fig. 3), though in such cases there is no evidence to show whether the hyperparasite can complete its development. The number of eggs laid by one female appeared to be about thirty. Only one egg is inserted at each oviposition, and others, when found, are probably the result of subsequent attacks.

The female Cynipid runs over the plant in an excited manner, vibrating her wings and tapping the aphides with her antennae. Healthy specimens are ignored, but the *Charips* seems to detect the presence of the primary parasite unerringly. When she finds an aphid containing a suitable host, she leaps on to its back, facing the head, and clings there firmly, despite its struggles, like a rider controlling a restless horse. Sometimes she is thrown off, but returns repeatedly to the attack until the aphid is exhausted into passivity. The actual insertion of the ovipositor takes from two to six minutes. This leisurely procedure is not surprising when it is remembered that the cuticle and body-wall of the aphid must be pierced before the probing for the host can begin, and as the *Aphidius* larva lies among the mass of aphid embryos its location can be no easy matter. Even when found the mesenteron is so distended with food that the body cavity is correspondingly reduced;

and if the ovipositor of the hyperparasite were to be thrust the smallest degree too far, the egg would be inserted in the host's gut, and be lost at evacuation of the meconium.

THE EGG.

The egg (Text-fig. 1) is an oval body, 0.010 mm. \times 0.006 mm., with a short peduncle continuous with its long axis. The oogenesis was not observed, but immediately after oviposition a cloud of deeply-staining granules was visible at the posterior pole. This may represent the germ-cell determinant, or, as it has recently been termed by Silvestri, the oosoma. An oosoma in the eggs of phytophagous Cynipidae was first described by Weismann in *Rhodites rosae* as the 'Furchungskern'. Hegner (12) has demonstrated it in *Diastrophes nebulosus*, and Hogben (14) in *Synergus*. The latter says of the last-named species that the oosoma appears as 'a cloud of granules, more and more heavily staining, until the determinant resembles a spherical ball at the end of the egg'. On the other hand, an oosoma has so far not been seen in other forms, such as *Rhodites ignota*, *Neuroterus*, *Andricus*, and *Cynips Kolleri*.

The described eggs of Cynipidae are all pedunculated, and in certain gall-forming species the peduncle may be five or six times the length of the egg-body. Adler (1) first pointed out that the peduncle is situated at the anterior pole of the egg, which, according to him, differs in this respect from the eggs of other Hymenoptera Parasitica. He supposed that the function of the peduncle is respiratory, and he was supported in this view by Cameron (3), who observed that the species which have long peduncles are those which place their eggs where they cannot receive much oxygen from the plant, while in the spring generations of the same species, which oviposit in the leaves, it is usually short. Hegner considers the peduncle analogous to the two anterior processes of the egg of *Ranatra linearis*, described by Korshelt, which float out in the water from the plant-tissues within which the egg is placed.

The observations of Riley, quoted by Sharp (22), suggest, however, that the peduncle may have another function. He found that in the ovipositions of *Callirhytes clavula* and *Biorhiza nigra* only the peduncle is inserted into the plant at first, and that the fluids collect at the posterior end of the egg. 'The fluids are then gradually absorbed from this exposed position into the inserted portion of the egg, and by the time the leaves have formed . . . the egg-contents are all contained within the leaf-tissue.'

Pedunculated eggs also occur in certain Chalcids. The egg of *Leucospis gigas* is furnished with a hooked process, whose purpose is evidently to suspend it from the cocoon of the *Chalicodoma* bee upon which the larva is parasitic. Imms (17) found that the egg of *Blastothrix britannica*, a parasite of *Lecanium capreae*, has a peduncle which protrudes through the body-wall of the host. The tip of the process disappears, thus putting the cavity of the chorion into communication with the outside air like a siphon. Timberlake (26) says that the egg of *Microterys*, parasitic upon *Coccus hesperidum*, is formed by two bodies connected by a hollow stalk. The stalk, together with the smaller body, projects through the body-wall of the host, and apparently serves for the respiration of the egg and of the larva in the early stages. The egg of *Aphelinus mytilaspidis*, parasite of *Lepidosaphes ulmi* (16), has also a process which, however, never projects outside the body of the host; and this is also the case with the egg of *Comys infelix*, a parasite of *Lecanium hemisphaericum*, described by Embleton (4) as possessing a bifid process. Howard and Fiske (15) state that the peduncles of the eggs of *Schedius kuvanae* protrude through the chorion of the eggs of the gipsy moth in which they are deposited. It may be remarked that four of these cases are parasites of Coccidae, sedentary animals whose metabolism and oxygen content must be low in comparison with that of other insects. Eggs approaching the pedunculated form occur in *Eucyrtus aphidivorus*, *Ageniaspis fuscicollis*, &c., and here perhaps the

increase of the egg's surface, in proportion to its mass, may bear some relation to oxygen absorption.

There is no reason why the peduncle should not in some cases be respiratory, as supposed by Adler, and in others for attachment, as suggested by Riley. In certain instances it possibly serves both functions; but its reduction in *Charips* probably indicates that it has lost its use, whatever that may have been.

TEXT-FIG. 1.

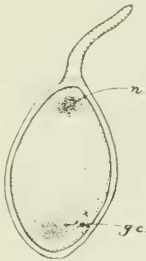


Fig. 1.—The egg immediately after oviposition. $\times 450$. *n.* = nucleus; *g.c.* = cloud of granules.

TEXT-FIG. 2.

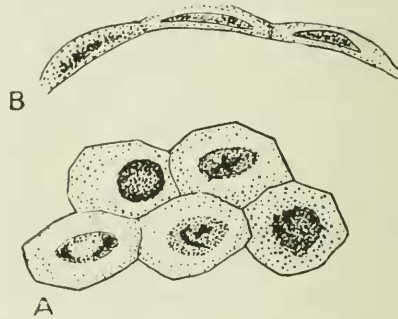


Fig. 2.—Cells of the trophic membrane with degenerating nuclei. A from above; B in section. $\times 350$.

THE EMBRYONIC MEMBRANE.

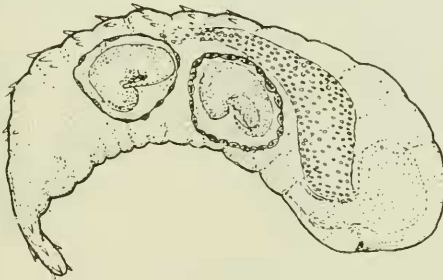
In *Charips*, as in certain other hymenopterous parasites, a trophic membrane or pseudoserosa is formed round the developing embryo as a globular sphere of large eosinophil cells, with definite nuclei and well-marked walls, polygonal in surface view and crescentic in section (Text-fig. 2). Membranes in this stage may be found up to the point of the hatching of the larva, after which they soon degenerate and disappear, though sometimes degeneration sets in at an earlier stage. A similar degeneration can be seen also in the membrane of the *Aphidius* host.

A membrane, resembling that described above, has been observed in certain Chalcids, but it does not appear to arise

in the same manner throughout the group. Silvestri (23, p. 67) has described its formation in *Encyrtus aphidivorus*, Mayr., where it originates as a delamination of the peripheral cells of the blastula. In the same work he gives an account of its origin in *Oophthora semblidis*, where at a certain point, the central protoplasm of the blastocoele breaks out through the blastoderm, bearing with it some free nuclei from the interior. This extruded protoplasm extends round the egg and forms the membrane.

In 1917 Gatenby (6) criticized the conclusions of Silvestri with regard to the latter species. Working on the development

TEXT-FIG. 3.



Larva of *Aphidius* containing two embryos of *Charips*. $\times 70$.

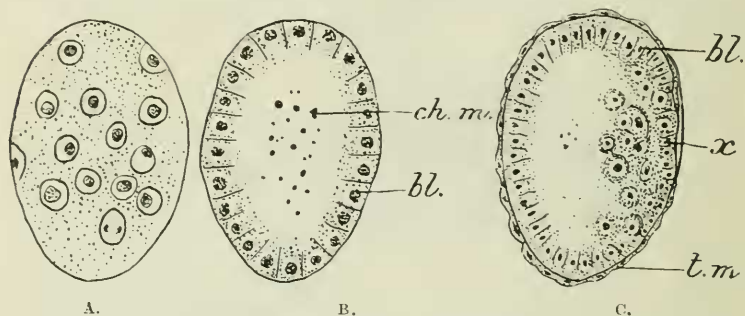
of *Trichogramma evanescens*, a form which he later recognized as con-generic with *Oophthora*, Gatenby showed that during the formation of the blastula small masses of nuclear matter are extruded into the blastocoele. Later, these, with the surrounding cytoplasm, move towards the periphery and ultimately stream out through the blastoderm. If the chorion is ruptured, the mass floats out into the host and soon perishes. If the chorion remains intact the extruded mass is flattened and extended by its pressure, until it surrounds the embryo, and the nuclei which it contains give it a fictitious cellular appearance.

Owing to the limited material at my disposal I originally intended to make no reference to the embryology of *Charips* ;

but in the course of this work three stages in the formation of the blastula were observed (Text-fig. 4), and therefore a partial description of them is now given.

A shows the egg soon after segmentation has begun. B represents the blastula already formed, and comparison with the figures of Silvestri and Gatenby shows no essential difference, save that in *Charips* the germ-cells are indistinguishable from the rest of the primary layer. In C the egg is seven hours old, and it will be seen that the nucleoplasmic masses

TEXT-FIG. 4.



Early stages in the segmentation of the egg. $\times 900$. *ch.m.* = extruded chromatin; *bl.* = blastoderm; *t.m.* = trophic membrane.

in the blastocoele have disappeared, and that there has been considerable displacement of the nuclei on the right-hand side. Certain nuclei are arranged in a manner that suggests that we have here a stage similar to that which Gatenby has indicated as the first appearance of the endoderm. Moreover, an involucre, apparently of cellular structure, surrounds the egg, and contains nuclear staining elements distinct from the degenerating chromatin masses shown in the previous figure. As intermediate stages are lacking it is impossible to say with certainty how this involucre arose.

Nearly all my available material was in the stage figured as B, but the membrane did not appear in it and there was no sign of the delamination described by Silvestri in *Encyrtus*.

Moreover, the arrangement of the cells does not suggest that they have arisen by division from the peripheral nuclei. The disappearance of the chromatin masses seems to indicate that there has been a recent escape of the contents of the blastocoele, but this matter does not appear in the involucre. It may be represented by a small mass found in the host's tissues opposite the point marked *x* in the figure. In any case, though Gatenby's explanation accounts for the appearance of the membrane in his own and in Silvestri's figures, it does not seem possible that the extruded matter could, under compression of the chorion, take an outline such as that shown in Text-fig. 4 c.

The data are too scanty to permit of our forming a definite opinion on the origin of the involucre in these Cynipidae, but I hope to pursue this subject later when more material is available. Gatenby, however, remarks that in some cases living nuclei are carried out with the extruded material: 'Curiously enough these fragments seem to live a good while, and nuclear changes, such as those undergone in the blastoderm, take place in some cases.'

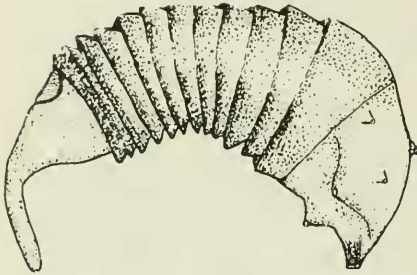
Without hazarding an opinion on the different views of these observers as regards the Trichogrammatinae, a suggestion may be made that if the expulsion of live nuclei were to be carried further in *Charips* than it is in *Trichogramma*, these might by division give rise to the membrane. But either this division must be very rapid, to develop the involucre in the space of two or three hours, or else the initial expulsion of the living nuclei must be larger than it appears to be from an examination of the material.

THE FIRST INSTAR (Text-fig. 5).

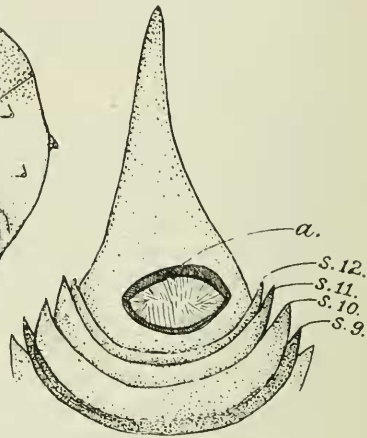
Dimensions, 0.38×0.13 mm. The embryonic membrane is ruptured two or three days after oviposition. The newly-hatched larva is heavily armoured with dark segmental plates of chitin, which render it easily visible through the tissues of the host. It possesses a distinct head and thirteen body-segments, the last of which terminates in a caudal appendage.

In the living larva the twelfth segment is somewhat telescoped into the eleventh, so that only twelve segments appear to be present. The mouth parts are produced into a proboscis, within which lie two long slender mandibles. The head bears three pairs of chitinous nodules on the ventral side, and, in addition, a fourth pair dorsally. These processes are each furnished at the extremity with a transparent spot which

TEXT-FIG. 5.



TEXT-FIG. 6.

Fig. 5.—Larva of the first instar. $\times 150$.Fig. 6.—Anus and caudal appendage of the newly-hatched larva.
 $\times 350$. *a.* = anus; *S.* 9-12 = chitinous plates of segments 9-12.

is possibly sensory in function. The body-segments diminish in diameter from the thorax posteriorly. Each appears as a circular band of chitin, somewhat overlapped by the one immediately preceding it. This overlap is so pronounced on the ventral side of the thorax in some examples as to give the effect of short processes; and as the latter actually appear after the first ecdysis it is possible that they may already exist under the chitinous plates, but at this stage it is not possible to demonstrate their presence definitely. The anus, which lies dorsal to the cauda, is a large and conspicuous structure surrounded by a chitinous ring (Text-fig. 6). From the

periphery transverse bands of chitin extend into the lumen, and give it a spiracle-like appearance. Owing to the opacity of the chitinous coat the internal organs cannot be seen, but the outline of the gut, which already contains food globules, is faintly visible by transparency.

The larva is curved ventrally with the tail bent round to form an angle with the abdomen. Its usual position is between the nerve-cord and gut of the host, either in the anterior or posterior third of the body. Owing to the manner in which the *Aphidius* lies in the aphid these are the parts most accessible to the ovipositor of the female *Charips*, and thus the earliest larval stage is presumably found where the egg has been deposited. The chitinized stage persists for a variable time. In one case observed the skin had been cast and left behind when the larva emerged from the trophic membrane. In others it lasted from two to four days. In the later stages the chitin can be found among the host's tissues. In ecdysis the skin usually splits transversely across the thorax, and the larva slips out. I have occasionally found examples in the second instar in which the moult had been incomplete, and the body of the larva was still encircled by one or more of the chitinous bands, like a rolled napkin enclosed by a ring.

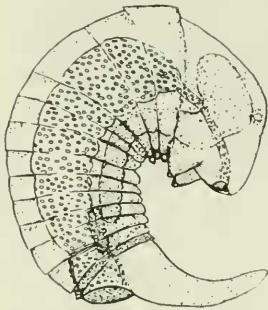
THE SECOND INSTAR (Text-fig. 7).

The second instar resembles the first in size and general form, but is white and transparent without thickened chitin. The mouth is transversely oval, and furnished with two large simple mandibles. Below it is a pair of ventro-lateral lobes surmounted by sensory papillae. Each of the three first body-segments bears a pair of protuberances on the ventral surface, and the segmentation of the body is less marked.

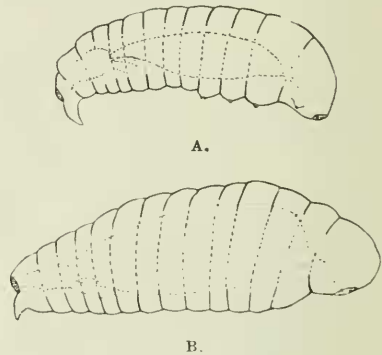
The internal structure is visible through the transparent integument. The salivary glands lie latero-ventrally on either side of the midgut as two straight tubes. The nerve-cord appears as a broad uncontracted band. The two Malpighian tubules are very short, and immediately behind their orifices

the proctodaeum is much enlarged with a bulb-shaped lumen, communicating with the exterior by the wide anus. In some examples newly removed from the host a transparent membranous substance was seen extruded from it. When larvae at this stage were stained with carmine or methylene blue, it was found that the stain readily entered through the anus, and was taken up by the lining epithelium of the hind-gut before any other part of the body was affected.

TEXT-FIG. 7.



TEXT-FIG. 8.

Fig. 7.—Larva of the second instar. $\times 150$.Fig. 8.—Intermediate stages of the larva. $\times 50$.

INTERMEDIATE STAGES (Text-fig. 8).

As the larva increases in size the tail and cephalic papillae become reduced, and the thoracic processes disappear. It was not ascertained whether there was a moult between this and the previous stage, or whether the change of form was due merely to growth and absorption of the appendages; but it is probable that there was at least one ecdysis about this time, though it was not actually observed. The body becomes much distended as the gut is filled with food matter, until the tail and processes finally vanish. After the disappearance of the cauda the anus gradually shifts back until it is at last terminal, and at the same time it becomes proportionately smaller.

The egg, as previously mentioned, is usually deposited in

the ventral side of the *Aphidius* at either extremity of the body. The chitinized larva, and subsequently its cast skin, are found in the same position, and orientated indifferently in any direction, but the later stages invariably lie along the dorsal side of the gut of the host with the head towards the head of the latter. Hence at some intermediate stage the hyperparasite must change its position. How this takes place was not observed, but, in view of the fact that the cauda of analogous forms is sometimes regarded as locomotory, it may be remarked that in *Charips* the first tailed larva does not move at all, while at some later stage, when the cauda is reduced, a definite, and frequently elaborate, change of position occurs.

THE FULL-GROWN LARVA (Text-fig. 9).

When the larva is full grown it makes its way out behind the head of the host, whose remains it devours within the next few hours. The gut may then be evacuated and metamorphosis ensue speedily, but frequently there is a resting period of several days. Thus, in one case, eleven days elapsed between emergence and transformation, and in another case, eight.

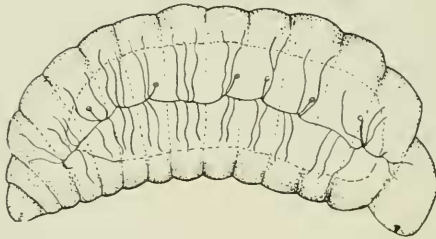
The full-grown larva is an apodous form measuring 1.70×0.90 mm. The body of thirteen distinct segments tapers somewhat to the anus. The skin is smooth, and there are no appendages except to the mouth parts. The crescentic labrum is furnished with eight small papillae. The mandibles are large, bidentate, and strongly chitinized. Each maxilla bears a disk, upon which are three papillae, one of which terminates in a short seta; and the labium, which is large and oval, bears laterally two pairs of papillae (Text-fig. 10).

The salivary glands, which in this form never secrete silk, extend forward from the seventh segment on either side of the gut ventrally. Each gland is a long straight tube composed of polyhedral cells, and, in the first segment, enters a duct which immediately behind the head unites with its fellow of the opposite side to form the short dilated common salivary duct

opening on the floor of the mouth under the U-shaped hypopharynx.

The mid-gut is shut off from the oesophagus by a valve. The former, which is greatly distended, is lined with flattened polyhedral cells with large nuclei. As in other hymenopterous larvae at this stage there is no communication between the

TEXT-FIG. 9.



TEXT-FIG. 10.

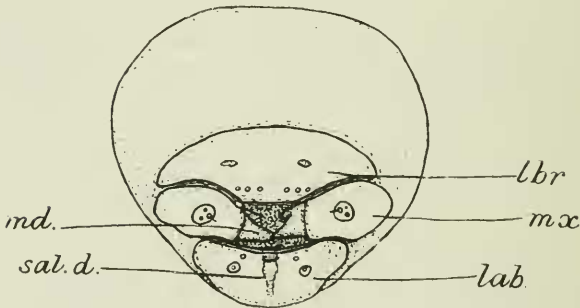


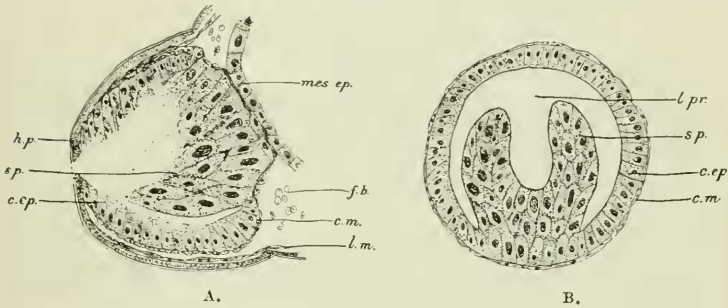
Fig. 9.—The full-grown larva. $\times 35$.

Fig. 10.—Head of full-grown larva. $\times 100$. *lab.* = labium; *lbr.* = labrum; *md.* = mandible; *mx.* = maxilla; *sal. d.* = salivary duct.

mesenteron and proctodaeum. The structure of the latter merits description. In larvae of the first and second instars the lumen is wide, and lined with a columnar epithelium of hypertrophied hypoderm cells with conspicuous nuclei. As development proceeds the anus becomes proportionately smaller, and an outgrowth from the antero-ventral wall of the proctodaeum projects backwards into the lumen. This outgrowth is shaped like a shovel, shortest on its dorsal aspect, and has

lateral expansions over-arching the cavity inside. In effect, it partly divides the proctodaeum into two compartments, one within the other, and the Malpighian tubules communicate ventrally with the inner of the two. The outgrowth or process itself is formed of two layers of elongated basophil cells, with well-marked nuclei, similar to those of the wall of the hind-gut, and in the later stages it almost fills the lumen. If it contained muscular fibres it would be easy to suppose that this outgrowth functions as a valve, shutting off the orifices of the Malpighian tubules from the general proctodeal cavity; but as the presence

TEXT-FIG. 11.



Proctodaeum of the young larva. A in sagittal, B in transverse section. $\times 350$. *c.ep.* = columnar epithelium; *c.m.* = circular muscles; *f.b.* = fat-body; *h.p.* = hypoderm; *l.m.* = longitudinal muscles; *l.pr.* = lumen of proctodaeum; *mes.ep.* = epithelium of mesenteron; *s.p.* = process projecting into the lumen of the proctodaeum.

of muscular tissue cannot be demonstrated, its only purpose appears to be to increase the surface area of the columnar epithelium of the hind-gut (Text-fig. 11).

The two Malpighian tubules are exceedingly short. Each is composed of eight or nine large cells only, but these surround a lumen of considerable diameter. The nervous system appears as a broad slightly-constricted band. The supra- and sub-oesophageal ganglia, and the three ganglia of the thorax, are well marked; but those of the abdominal region are indistinctly separated, with the exception of the last two, which are fused and form a distinct bulb-like swelling.

The rest of the internal structure demands no particular comment.

The tracheal system becomes functional when the parasite leaves the host. The two main lateral trunks are united by an anterior and a posterior commissure. Dorsal and ventral lateral branches are given off in each segment 1-10. There are six pairs of open spiracles. The first is placed between segments 1 and 2, and the remainder on segments 3, 4, 5, 7, and 9. Of the considerable number of examples examined only two departed from this rule in possessing, in addition, a pair of spiracles on segment 8.

PUPATION AND EMERGENCE.

Pupation lasts from twenty-two to twenty-six days, and at the end of this time the Cynipid gnaws an irregular hole on the dorsal side of the cocoon and creeps out. In captivity the adults lived from three to eight days. They fed upon the sap oozing from cut leaves and upon the honeydew of the aphides. They sometimes sipped the latter from the anus of the living animal, and were occasionally observed to scrape the dried sugar from empty skins with their mandibles.

It is not known how many broods may be reared in the season, nor how far these Cynipid hyperparasites are specific for different Aphidiidae, but as far as it goes the evidence suggests that they have a considerable range of hosts. Thus the number of broods is probably determined by the number of Aphidiidae available.

Also at present there is no evidence as to how the parasites and hyperparasites of Aphides pass the winter. I have found living larvae of *Aphidius salicis*, Hal., in *Aphis saliceti*, Kalt., in cocoons collected in July, and opened in the laboratory in January. This suggests that a few may pass through the winter in this stage; but, although I paid particular attention to this point, I could find no indication that *Aphidius ervi* had not all emerged by the end of August, for, of the considerable number of cocoons from different localities that were examined, all were empty.

COMPARISON OF THE LARVAL CHARACTERS OF *Charips* WITH THOSE OF OTHER ENTOMOPHAGOUS CYNIPIDAE.

Our knowledge of the larval forms of the other entomophagous Cynipidae is limited to three species.

In 1834 Bouché (2) described the full-grown larva of *Figites anthomyiarum*, Bouché, found in the puparia of *Anthomyia* (Diptera).

In 1886 Handlirsch (9) gave an account of the corresponding stage of another Figitine, *Anacharis typica*, Walker, parasitic upon *Hemerobius nervosus*, Fabr.

In 1913 Keilin and Pluvinel (18) described the post-embryonic development of an Encoiline, *Encoila keilini*, Kieff., parasitic upon the Dipteron *Pegomyia*.

In comparing the full-grown larva of *Charips* with these three forms, we find certain structural differences between them. *Charips* and *Anacharis* possess thirteen segments, whereas *Figites* and *Encoila* have but twelve. The tubercles of *Anacharis* are distinctive, and *Encoila* alone possesses simple mandibles. *Figites*, *Anacharis*, and *Encoila* all have nine pairs of spiracles, a character they share with the phytophagous forms. In *Charips* there are but six pairs of spiracles (exceptionally seven), and these are not arranged upon consecutive segments.

As regard the early stages, the only form available for comparison with *Charips* is *Encoila*. In the first instar the larvae are of the same general type, but *Charips* differs from *Encoila* in the absence of pronounced thoracic processes, and in the possession of a chitinized skin, mandibles, and an enlarged anus. The embryonic membrane does not seem to occur in *Encoila*, and, so far, has not been recorded in the Cynipidae.

COMPARISON OF THE LARVAL CHARACTERS OF CHARIPS
WITH THOSE OF PARASITIC HYMENOPTERA IN GENERAL.

The early larvae of the hypermetamorphic Hymenoptera Parasitica may be referred to three main groups:

The first, or cyclopid, type so far as has been found only in *Platygaster* (Proctotrypoidea), and is known chiefly through the researches of Ganin (5) and Marchal (20).

In the second type the last segment is furnished with an appendage, and thus may be called caudate. It includes, for example, such forms as *Limnerium* (Ichneumonidae) *Aphidius* (Braconidae), *Comys*, and certain *Ageniaspid*s (Chalcidae) and *Teleas* (Scelionidae).

The third type was first observed by Wheeler (27) in the myrmecophilagous Chalcid, *Orasema*, and has since been described by Smith (24) in another Chalcid, *Perilampus*. This larva, known as a planidium, is elongated and testudinate, furnished with imbricated plates of chitin.

The caudate type is the most frequent. The function of the tail has been supposed by different authors to be either locomotory or respiratory, but may possibly be both. In the early stages of such forms the tracheal system is apneustic and respiration is cutaneous. The cauda, by increasing the body-surface, may assist in the absorption of oxygen, and the thoracic processes of *Enecoila* may have a similar function. At the same time the setae with which the cauda is furnished in some Aphidiidae suggest that it may sometimes serve for locomotion.

The first-stage larva of *Charips* is caudate, but I can find no other instance of heavy chitinization in this type. Indeed, the only parallel instance appears to be the planidium of *Perilampus*, whose life-history is somewhat different. *Perilampus* is hatched as a free living form, and later seeks out the caterpillar which contains the proper hymenopterous or dipterous host. It then lives as an endoparasite without growth or ecdysis for a variable time. After metamorphosis of the host, it emerges, sheds its chitinized skin, and completes development

as an ecto-parasite upon the pupa. Here presumably the chitin protects the larva during the search for the host. *Charips* is an endoparasite throughout larval life, but certain facts suggest that this may be a later adaptation, and that the chitinous armour may be a survival of a life-cycle not unlike that of *Perilampus*.

For instance, the chitin does not now seem to be of vital importance to the young larva, since it may either be thrown off at hatching and left behind in the embryonic membrane or persist for a variable number of days afterwards. Smith (24) suggests that the histolysis of the surrounding tissues is the stimulus that impels the *Perilampus* to change its mode of life and moult. Something of the kind may occur in *Charips*, though in this form metamorphosis of the host does not actually take place. The host larvae may be in different stages of development at oviposition, and yet those younger than the third instar could scarcely contain enough food material to enable the Cynipid to reach maturity. It is doubtful whether in such a case as that shown in fig. 3, where the gut is already displaced before the hyperparasites have left the embryonic membrane, the *Aphidius* can survive. But even in ovipositions in third-instar Braconids it would be fatal to the Cynipid if the development of the host were arrested too soon, for instance before the cocoon was woven. Thus it is possible that the chitinized stage is in some sort a resting phase, and I now regret that I did not pay more attention to this point in the material at my disposal.

Another point is that *Perilampus* is endoparasitic only in the first instar, whereas *Charips* lives internally until larval development is completed.

But a parallel may be drawn if the internal habit of the latter is a comparatively recent adaptation, and the demolition of the host's remains after emergence is a survival from a time when it made its way out of the host at an earlier stage and completed development as an ectoparasite.

The metabolism of *Charips* presents certain problems. The thick chitin must prevent cutaneous transfusion of oxygen

from the host's tissues. It is possible that the structure of the anus and proctodaeum is correlated with this, and that something analogous to rectal respiration exists in this form. The hind-gut has a large lumen enclosed by modified hypoderm cells. In the later stages the proctodaeum is proportionately smaller, and, when the chitin is cast off, respiration is presumably carried on through the cuticle, as in such forms as *Aphidius*, though mention should be made of the tongue-shaped process of large deeply-staining cells, which, like a typhlosole, projects into the lumen of the proctodaeum as development proceeds, and, if the view suggested here is correct, would increase the respiratory area.

A peculiar modification of the hind-gut occurs in the larvae of certain Braconids, such as *Apanteles* and *Microgaster*. The body terminates in a hollow bladder or vesicle of hypertrophied cells; and Gatenby (8), who has recently re-described this structure, makes the interesting suggestion that this is morphologically the proctodaeum, which has become everted for respiration. The enlarged, though uneverted, hind-gut of *Charips* may be intermediate between the highly-specialized structure found in these *Microgasterinae* and the unmodified proctodaeum of most hymenopterous larvae.

It is noteworthy that in these Cynipidae great development of chitin is associated with unusually short Malpighian tubules. If the chitin persisted throughout larval life we might be tempted to regard it as a means of disposing of such nitrogenous waste material as could not be dealt with by the tubules. But as the chitinized plates are lost early, while the tubules do not increase in size in the later stages, it is improbable that the two characters are correlated.

REACTION OF THE HOST.

Aphidius reacts very differently to *Charips* and to *Lygoceus*. In parasitization by the latter, as described elsewhere (10), the host dies, and speedily deliquesces into a mass. Nothing of this kind happens where the Braconid contains a *Charips* larva. The *Aphidius* demolishes

the viscera of the aphid, and then secretes silk and weaves the cocoon as usual. The tissues retain their tone and colour, and irritation excites slight movement. On close examination, however, it can be seen that the body is somewhat contracted.

At this time the Cynipid larva, its head orientated with that of the host, lies above the mesenteron of the latter, which it constricts into a dumb-bell form. By some means the further development of the *Aphidius* is arrested, and always at the same point, namely, after the weaving of the cocoon. The meconium is never evacuated, and metamorphosis, which normally takes place soon afterwards, never occurs. The condition of the Braconid larva resembles in fact that of the prey that certain Hymenoptera store in their brood-cells.

Two explanations of this phenomenon suggest themselves. Either the female *Charips* at oviposition may inhibit the final changes of the host, possibly by injection of some secretion; or the Cynipid larva itself, during development, may affect the *Aphidius* by chemical or physical means.

The evidence is not conclusively in favour of either view. In support of the first one particularly marked instance came under notice.

A *Charips* female was observed to oviposit on June 26. The aphid was isolated, and four days later the *Aphidius* within began to spin silk. On July 4 the cocoon was opened in order better to follow the development of the hyperparasite, a plan that was adopted successfully in several instances. The *Aphidius* remained without change until August 7, a period of five weeks. The meconium was not voided, but beyond some contraction the larva looked healthy. In replacing it in the tube after examination it fell from the brush, and must have received some injury, for next day a discoloured patch appeared at the hinder end of the body. The larva was dissected carefully, but no hyperparasite could be found, and the organs showed little signs of histolysis. As oviposition had been observed, the facts suggest that some accident had prevented the development of the Cynipid larva, and this leads to the inference that the agent arresting the metamorphosis of the

host comes into force, if not at oviposition, at least at an early stage in larval life.

In support of the view that the larva itself may inhibit the development of the host is the parallel case of *Perilampus*. As the larva is hatched as a free-living form and subsequently enters the host, there can be no question of the inhibition dating from oviposition. Yet, according to Smith (24), 'The development of the host . . . invariably ceases at the time of exit of the planidium. Whether or no it is actually killed is not evident. In any case decomposition does not take place immediately, the host being left in a condition somewhat comparable to that of the prey of certain aculeate Hymenoptera.'

Perilampus differs from *Charips* in that metamorphosis has taken place before the exit of the planidium; but when the latter begins to live as an ectoparasite upon the newly-formed pupa, it is found that the growth of the head and appendages, with their setae and pigments, is arrested, and development is not completed.

Nothing resembling phagocytic reaction against the hyperparasite was observed, either as regards the living larva or the cast skin, which could sometimes be found unchanged among the host's tissues up to the time of emergence of the full-grown Cynipid larva.

ECONOMIC STATUS.

Charips checks the *Aphidius* in its destruction of plant-lice, and thus, from the economic standpoint, must be considered an injurious insect. But throughout its development it shares the vulnerability of its host to ectoparasitic Chalcids and Proctotrypids, and when secondary parasitization occurs it perishes with the *Aphidius*. From observations made in the course of this work it would seem that where the incidence of Chalcid and Proctotrypid hyperparasitization is high, the chances of *Charips* larvae attaining maturity are correspondingly reduced. For instance, if, of a hundred *Aphidius*, twenty-five are parasitized by *Charips*, and thirty-two

parasitized by such a form as *Lygocerus* (Proctotrypidae) by chance, 8 per cent. of the former should be destroyed; while where the incidence of parasitization by Chalcids, such as *Asaphes*, is as high as that of *Lygocerus*, this rate of mortality must be doubled. The above figure for Cynipidae is hypothetical, though, as it is based on examination of much material, it is probably not too low. That for *Lygocerus* was found to be the actual rate in certain instances (10). It is difficult to estimate the mortality accurately, because the host, if subsequently reparasitized, rapidly decomposes, and any endoparasite that it may contain soon becomes unrecognizable. Moreover, the bionomical relations of the different hyperparasites are so intricate that the chances of survival of any particular case are difficult to compute. Thus *Charips* actually lessens its own chance of survival, for the effect of its parasitization is to arrest the metamorphosis of the host, and thus maintain it in the optimum condition for oviposition by *Lygocerus* or *Asaphes*. Hence in the hypothetical case given above the number of *Aphidius* larvae parasitized by *Charips* and reparasitized by *Lygocerus* would probably be larger than that parasitized by *Lygocerus* only, and the mortality of the first parasite would actually be higher than the figure given. To this mortality from reparasitization I attribute the fact that from collections of parasitized aphides made in the field there were proportionately more Cynipid emergences in June than in July. Most of the hyperparasites obtained from later collections were Chalcids or Proctotrypids (*Lygocerus*); and the inference is that the later broods of Cynipidae suffered from a second parasitization of their hosts by other hyperparasites.

SUMMARY.

1. *Bothryoxysta curvata*, Kieff., *Charips vietrix*, Hartig, and *Alloxysta erythrothorax*, Westw., are hyperparasites of aphides through *Aphidius* (Braconidae).
2. Reproduction may be either sexual or parthenogenetic.
3. The egg is laid in the haemocoel of the host larva before

the death of the aphid, and post-embryonic development is internal.

4. A trophic membrane of hypertrophied cells is formed round the embryo.

5. The larva is, at first, hypermetamorphic; and exhibits greater development of the chitinous cuticle than is usual in endoparasites; but in the succeeding stages it approximates more closely to the general hymenopterous type.

6. The development of the *Aphidius* is arrested at a certain point, and metamorphosis does not take place.

7. The Cynipid, when ready to pupate, makes its way out of the *Aphidius*, whose remains it devours, and undergoes metamorphosis within the cocoon previously woven by the latter in the skin of the aphid.

8. These forms differ in certain particulars from the entomophagous Cynipidae previously described, and the chief differences are discussed.

9. Comparison is also made of the larvae of other Hymenoptera Parasitica, particularly of *Perilampus*.

10. Certain problems of metabolism are pointed out, and it is suggested that respiration may be partly rectal.

11. These Cynipidae are economically injurious as they check the *Aphidius*'s in its destruction of plant-lice; but there is high mortality among the larvae owing to secondary parasitization of the Braconid by other hyperparasites.

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